

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>ONTCA-49B</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/CA 98/ 01137</b>	International filing date (day/month/year) <b>08/12/1998</b>	(Earliest) Priority Date (day/month/year) <b>04/02/1998</b>
Applicant <b>THE ONTARIO CANCER INSTITUTE et al.</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 7 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
  - the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).
- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :
  - contained in the international application in written form.
  - filed together with the international application in computer readable form.
  - furnished subsequently to this Authority in written form.
  - furnished subsequently to this Authority in computer readable form.
  - the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
  - the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2.  Certain claims were found unsearchable (See Box I).

3.  Unity of invention is lacking (see Box II).

4. With regard to the title,

- the text is approved as submitted by the applicant.
- the text has been established by this Authority to read as follows:

5. With regard to the abstract,

- the text is approved as submitted by the applicant.
- the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

- as suggested by the applicant.
- because the applicant failed to suggest a figure.
- because this figure better characterizes the invention.

1b

None of the figures.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 98/01137

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
**Remark:** Although claims 17, 24 and 25 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 98/01137

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/10 C07K14/25 C12Q1/68 A61K31/70 A61K39/02  
G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K C12Q A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	T. IIDA ET AL.: "A single amino acid substitution of Escherichia coli enterotoxin affects its oligomer formation" J. BIOL. CHEM., vol. 265, no. 24, 25 August 1989, pages 14065-14070, XP002096323 AM. SOC. BIOCHEM. MOL.BIOL., INC., BALTIMORE, US see Materials and Methods see page 14068, right-hand column, paragraph 2 ---	1,3,5,8, 9,16,17
Y	see page 14068, right-hand column, paragraph 2 ---	1,6 -/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## ° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

12 March 1999

26/03/1999

Name and mailing address of the ISA

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 98/01137

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	C. CLARK ET AL.: "Phenylalanine 30 plays an important role in receptor binding of verotoxin-1" MOLECULAR MICROBIOLOGY, vol. 19, no. 4, February 1996, pages 891-899, XP002096376 BLACKWELL, OXFORD, GB cited in the application see the whole document ---	1,3,5,8, 9,16,17
X	G.J. TYRELL ET AL.: "Alteration of the carbohydrate binding specificity of verotoxins from Gal(alpha)1-4Gal to GalNAc(beta)1-3Gal(alpha)1-4Gal and vice versa by site-directed mutagenesis of the binding subunit " PROC. NATL. ACAD. SCI., vol. 89, January 1992, pages 524-528, XP002096324 NATL. ACAD. SCI., WASHINGTON, DC, US; cited in the application see the whole document ---	17,18,21
Y	L.P. PERERA ET AL.: "Identification of three amino acids residues in the B subunit of Shiga toxin and Shiga-like toxin type II that are essential for holotoxin activity" J. BACTERIOL., vol. 173, no. 3, February 1991, pages 1151-1160, XP002096325 AM. SOC. MICROBIOLOGY, BALTIMORE, US; cited in the application see the whole document ---	1,6 1-3,5, 8-10, 16-18,21
Y	D.J. BAST ET AL.: "Toxicity and immunogenicity of a verotoxin 1 mutant with reduced globotriaosylceramide receptor binding in rabbits" INFECTION AND IMMUNITY, vol. 65, no. 6, June 1997, pages 2019-2028, XP002096326 ASM, WASHINGTON, DC, US see the whole document ---	1,6 17,18
Y		1,6
		-/-

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 98/01137

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S.W. LINDGREN ET AL.: "The specific activities of Shiga-like toxin type II (SLT-II) and SLT-II related toxin of enterohemorrhagic Escherichia coli differ when measured by vero cell cytotoxicity but not by mouse lethality" INFECTION AND IMMUNITY, vol. 62, no. 2, February 1994, pages 623-631, XP002096327 ASM, WASHINGTON, DC, US see the whole document ---	1-3,5, 8-10, 16-18,21
X	M.P. JACKSON ET AL.: "Functional analysis of the Shiga toxin and Shiga-like toxin type II variant binding site subunits by using site-directed mutagenesis" J. BACTERIOL., vol. 172, no. 2, February 1990, pages 653-658, XP002096328 AM. SOC. MICROBIOL., BALTIMORE, US; see the whole document ---	1-3,5, 8-10, 16-18,21
Y	J.D. MERMES ET AL.: "A reliable method for random mutagenesis: the generation of mutant libraries using spiked oligonucleotide primers" GENE, vol. 84, 1989, pages 143-151, XP002096329 ELSEVIER SCIENCE PUBLISHERS, B.V., AMSTERDAM, NL; cited in the application see the whole document ---	1
Y	G. DEL RÍO ET AL.: "Combinatorial libraries of proteins: Analysis of efficiency of mutagenesis techniques" BIOTECHNIQUES, vol. 17, no. 6, 1994, pages 1132-1139, XP002096330 NATICK, MA, US cited in the application see the whole document ---	1,6
A	A.A. KHINE AND C.A. LINGWOOD: "Capping and receptor-mediated endocytosis of cell-bound verotoxin (Shiga-like toxin) 1: Chemical identification of an amino acid in the B subunit necessary for efficient receptor glycolipid binding and cellular internalization" J. CELL PHYSIOL., vol. 161, no. 2, November 1994, pages 319-332, XP002096331 Wiley-Liss, Inc., new york, US see the whole document ---	1-26

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International Application No

PCT/CA 98/01137

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	P.-G. NYHOLM ET AL.: "Two distinct binding sites for globotriaosyl ceramide on verotoxins: identification by molecular modelling and confirmation using deoxy analogues and a new glycolipid receptor for all verotoxins" CHEMISTRY AND BIOLOGY, vol. 3, no. 4, April 1996, pages 263-275, XP002096332 Current Biology LTD, ISSN 1074-5521 cited in the application see the whole document ---	1-26
A	P.G. NYHOLM ET AL.: "Modelling of the interaction of verotoxin-1 (VT1) with its glycolipid receptor. globotriaosylceramide (Gb3)" INT. J. BIOL. MACROMOL., vol. 17, no. 3-4, June 1995, pages 199-204, XP002096333 Elsevier Science B.V., Amsterdam, NL cited in the application see the whole document ---	1-26
A	P.E. STEIN ET AL.: "Crystal structure of the cell-binding B oligomer of verotoxin-1 from E.coli" NATURE, vol. 355, 20 February 1992, pages 748-750, XP002096334 MACMILLAN JOURNALS LTD., LONDON,UK cited in the application see the whole document ---	1-26
P,X	BRAY, M. R. ET AL: "Shiga-like toxin as a template for the development of anti-breast cancer agents." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, (MARCH, 1998) VOL. 39, PP. 62-63. MEETING INFO.: 89TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH NEW ORLEANS, LOUISIANA, USA MARCH 28-APRIL 1, 1998 AMERIC, XP002096335 Abstract no. 428; --- -/-	1-17

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 98/01137

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	<p>BRAY, M. R. ET AL: "Expression of the Shiga-like toxin I receptor CD77 on human breast carcinomas, follicular lymphomas and multiple myelomas and absence of expression on CD34+ human hematopoietic cells: Implications for tumor cell purging."</p> <p>PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, (MARCH, 1998) VOL. 39, PP. 63. MEETING INFO.: 89TH ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH NEW ORLEANS, LOUISIANA, USA MARCH 28-APRIL 1, 1998 AMERICAN , XP002096336 Abstract no. 429;</p> <p>---</p>	18,21
P,A	<p>H. LING ET AL.: "Structure of the Shiga-like toxin I B-pentamer complexed with an analogue of its receptor Gb3"</p> <p>BIOCHEMISTRY, vol. 37, 17 February 1998, pages 1777-1788, XP002096337</p> <p>AM. CHEM. SOC., WASHINGTON, DC, US see the whole document</p> <p>-----</p>	1-26